Received: 6 December 2019 | Revised: 28 June 2020 | Accepted: 9 July 2020

DOI: 10.1111/sms.13776

ORIGINAL ARTICLE

WILEY

Caffeine increases strength and power performance in resistance-trained females during early follicular phase

Martin Norum[1] | **Linn Christin Risvang**[1,2] | **Thomas Bjørnsen**[3,4] | **Lygeri Dimitriou**[1,5] | **Per Ola Rønning**[2] | **Morten Bjørgen**[6] | **Truls Raastad**[7]

[1]School of Science and Technology, London Sport Institute, Middlesex University, London, UK

[2]Department of Mechanical, Electronics and Chemical Engineering, Faculty of Technology, Art and Design, Oslo Metropolitan University, Oslo, Norway

[3]Department of Sport Science and Physical Education, Faculty of Health and Sport Sciences, University of Agder, Kristiansand, Norway

[4]Norwegian Olympic and Paralympic Committee and Confederation of Sports, Oslo, Norway

[5]Department of Natural Sciences, School of Science and Technology, Middlesex University, London, UK

[6]Department of Life Sciences and Health, Faculty of Health Sciences, Oslo Metropolitan University, Oslo, Norway

[7]Department of Physical Performance, Norwegian School of Sport Sciences, Oslo, Norway

Correspondence
Linn Christin Risvang, Department of Mechanical, Electronics and Chemical Engineering, Faculty of Technology, Art and Design, Oslo Metropolitan University, Oslo, Norway.
Email: linnrisv@oslomet.no

The effects of 4 mg·kg^{-1}caffeine ingestion on strength and power were investigated for the first time, in resistance-trained females during the early follicular phase utilizing a randomized, double-blind, placebo-controlled, crossover design. Fifteen females (29.8 ± 4.0 years, 63.8 ± 5.5 kg [mean ± SD]) ingested caffeine or placebo 60 minutes before completing a test battery separated by 72 hours. One-repetition maximum (1RM), repetitions to failure (RTF) at 60% of 1RM, was assessed in the squat and bench press. Maximal voluntary contraction torque (MVC) and rate of force development (RFD) were measured during isometric knee extensions, while utilizing interpolated twitch technique to measure voluntary muscle activation. Maximal power and jump height were assessed during countermovement jumps (CMJ). Caffeine metabolites were measured in plasma. Adverse effects were registered after each trial. Caffeine significantly improved squat (4.5 ± 1.9%, effect size [ES]: 0.25) and bench press 1RM (3.3 ± 1.4%, ES: 0.20), and squat (15.9 ± 17.9%, ES: 0.31) and bench press RTF (9.8 ± 13.6%, ES: 0.31), compared to placebo. MVC torque (4.6 ± 7.3%, ES: 0.26), CMJ height (7.6 ± 4.0%, ES: 0.50), and power (3.8 ± 2.2%, ES: 0.24) were also significantly increased with caffeine. There were no differences in RFD or muscle activation. Plasma [caffeine] was significantly increased throughout the protocol, and mild side effects of caffeine were experienced by only 3 participants. This study demonstrated that 4 mg·kg^{-1} caffeine ingestion enhanced maximal strength, power, and muscular endurance in resistance-trained and caffeine-habituated females during the early follicular phase, with few adverse effects. Female strength and power athletes may consider using this dose pre-competition and -training as an effective ergogenic aid.

KEYWORDS

caffeine supplementation, female athletes, muscular activation level, muscular endurance, strength and power performance

Martin Norum and Linn Christin Risvang should be considered joint first authors.

1 | INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is the most widely used legal drug in the world, by the general as well as athletic populations,[1] and researchers' interest in the effects of caffeine on exercise performance is apparent in light of multiple reviews of the literature published in the recent years.[2-5] These reviews currently agree that caffeine is a potent ergogenic aid for a variety of exercise performances; however, the effects of caffeine on maximal strength and power performance are less clear. Meta-analyses by Warren et al[6] and Polito et al[7] showed that caffeine ingestion can increase isometric strength and muscular endurance performance. However, Polito et al[7] could not observe improved dynamic strength with caffeine supplementation, and a recent meta-analysis by Grgic et al[4] only found increased performance in upper but not lower body dynamic strength. On the other hand, increased muscular endurance has been demonstrated with larger effect sizes in lower body rather than upper body exercises.[7] The conflicting results could be due to varying effect of caffeine on different types of contractions, as the contribution of cortical and spinal centers to the neural drive changes with the contraction type.[8] Hence, further research is warranted to investigate the effect of caffeine on maximal isometric versus dynamic strength, power, and muscular endurance, as well as comparing lower and upper body muscle groups.

A recent review of the caffeine literature found that only ~13% of the total sample in research on the ergogenic effect of caffeine between 1978 and 2018 were women and that the number of women in studies investigating caffeine effects on speed and muscle power is very low.[9] A likely explanation for this difference in representation of the sexes is that females can be a slightly more challenging cohort to conduct caffeine research on. The use of oral contraceptives[10] and the large variations in hormone concentrations between phases of the menstrual cycles[11] can alter caffeine metabolization speeds,[12] which in turn may alter the ergogenic effects of caffeine. Indeed, significant sex differences have been reported in caffeine concentrations post-exercise with ingestion of 3 mg/kg caffeine, with females having a greater amount. This suggests that females do not metabolize caffeine as rapidly as males. Furthermore, variations in strength and power have been demonstrated throughout the menstrual cycle,[13] which can cause noise in performance data and affect overall results. Taken together, although there are a number of studies demonstrating that caffeine clearly has an ergogenic effect in females,[9,14-16] the information about the effect of caffeine on muscle performance in women is uncertain, especially in strength and power performance. As an example, a recent meta-subgroup analysis examined the effects of caffeine on muscle power in females for the first time.[4] However, only three studies examining vertical jumps were included and neither controlled for potential metabolic alterations across the menstrual cycle, making it difficult to conclude on the effects of caffeine on power in females. Moreover, a recent study found differences in the effect of caffeine on power performance between the phases in the menstrual cycle.[17] It, therefore, seems important to control for stages in the menstrual cycle to further establish clear recommendations for the use of caffeine in females. The early follicular phase of the menstrual cycle has shown the lowest variability in oestradiol and progesterone concentration,[18] and the sex hormone levels in this phase are similar to the levels in females using hormone contraceptives.[19] Furthermore, a recent study found that the fluctuations in sex hormones throughout the menstrual cycle affect neuromuscular function.[20] Hence, conducting caffeine research on females would benefit from being performed at the same stage of the menstrual cycle and can reliably be performed during the early follicular phase.

The underlying mechanisms by which caffeine may aid maximal strength and power are likely increased motor unit recruitment and voluntary muscle activation of the involved muscles.[6,21,22] However, there seem to be discrepancies in the caffeine effect on strength and power that corresponds to varying degree of baseline voluntary activation. Larger lower body muscles such as knee extensors seem to have a relatively low (85%-95%) muscle activation level compared to the small upper body muscles (90%-99%),[6] such as elbow flexors.[23] These differences in baseline muscle activation may influence the magnitude of the caffeine effect. As Warren et al[6] discuss in their meta-analysis, logically there will be more to improve with lower baseline muscle activation levels, that is, larger lower body muscles might have a greater effect of caffeine. Correspondingly, strength and power improvements with caffeine have been reported in this pattern.[6] However, one study in females shows the quite opposite pattern, that is, caffeine-induced improvements of upper body but not lower body maximal strength, although this needs further investigation.[16] Perceived pain and exertion during exhaustive resistance work have been thought to be reduced, and thereby improving performance, through caffeine's inhibitory binding to adenosine receptors.[21] However, caffeine's effect on intra-set ratings of perceived exertion seems under-investigated compared to post-fatigue ratings, although Doherty et al's meta-analysis[24] observed that a ~5% reduction in intra-set ratings of perceived exertion (RPE) explained about a third of the variance in exhaustive work between caffeine and placebo. Moreover, the contribution of muscle activation to increased strength and power, comparison of upper and lower body maximal strength and effects on RPE and pain has to the authors' knowledge, not been investigated specifically with moderate caffeine doses in resistance-trained females while controlling for menstrual cycle.

Only three studies have investigated the effects of caffeine doses <6 mg·kg^{-1} on strength performance in females.[16,25,26] Goldstein et al[27] and others[4] have specifically proposed that future research should examine the ergogenic effects of lower doses of caffeine. Several studies have reported severe side effects such as "intense emotional responses," tremor, heart palpitations, and tachycardia when supplementing with relatively high doses of caffeine (6-11 mg·kg^{-1}).[27-29] A lower caffeine dose could induce similar performance enhancements but with fewer adverse events, which would be an advantage, especially to competing strength and power athletes.

Thus, the main purpose of the present study was to investigate, for the first time, the effects of 4 mg·kg^{-1} caffeine on various strength and power measures in resistance-trained females during the early follicular phase. We hypothesized a caffeine-induced increase in maximal strength and muscular activation levels, vertical jump height, as well as in muscular endurance, compared to placebo ingestion. Secondary outcomes of the study were intra-set ratings of perceived exertion, perceived pain, plasma caffeine concentration, habituation, and adverse effects.

2 | METHODS

2.1 | Participants

Fifteen caucasian female volunteers (age: 29.8 ± 5.5 years; stature: 165.8 ± 4.8 cm; body mass: 63.8 ± 5.5 kg [mean ± SD]) completed this study (Table 1). Nine of the 25 recruited participants dropped out after randomization due to logistical issues, and one was excluded due to intake of a source of caffeine unknown to participant and researchers. Resistance-trained participants (recreational lifters, personal trainers, and functional fitness athletes) were recruited following these inclusion criteria: (a) 18-45 years old; (b) resistance-trained for minimum 12 months, 2-3 sessions/week and currently resistance training; (c) ability to perform squat and bench press with a load corresponding to 110% and 80% of their current body mass, respectively, and (d) familiar with the bench press and back squat exercises (performed at least one time/wk). Participants were excluded if they were smokers, pregnant, or lactating, were adversely affected by caffeine, used medicines and/or other ergogenic supplements, had history of recent injury, illness or other diseases that could affect measurements. Participants signed a written informed consent and completed a Physical Activity Readiness Questionnaire (PAR-Q). Ethical approval was obtained from the research ethics committee of London Sports Institute, Middlesex University (London, UK) and the Norwegian School of Sport Science (Oslo, Norway). The project was approved by the Norwegian Centre for Research Data.

2.2 | Study design

A randomized, double-blind, placebo-controlled crossover design was used to investigate the effects of 4 mg·kg^{-1} caffeine on strength and power performance. The participants attended four sessions; two familiarizations to all procedures (except blood sampling) and to the test battery, and two trials. However, three familiarization sessions were performed when the variation between the two first familiarization sessions exceeded a coefficient of variation (CV) of 10% (total number of participants completing three familiarizations for one of the tests, n = 8). Participants were instructed to refrain from alcohol, caffeine, and vigorous physical activity 48 hours prior to the trials and were provided with a detailed list of items containing caffeine, such as coffee, chocolate, tea, soda, and energy drinks. All participants recorded their weekly intake of these products using a caffeine frequency questionnaire to calculate their habitual caffeine intake (Table 1), and were classified as low, medium, or high caffeine consumers based on habitual intakes (<1.5, 1.5-5.0 and >5.0 mg·kg^{-1}·d^{-1}, respectively).[30] They also completed a 24-hour food diary (MyFitnessPal®, MyFitnessPal, Inc) prior to the first trial and replicated the food intake prior to the second trial to ensure minimal variation in hydration level and energy intake. Body composition was assessed by bioelectrical

TABLE 1 Participant characteristics.

	Mean ± SD	Range
Fat-free mass (kg)[a]	52.3 ± 5.2	44.4-63.2
Fat mass (kg)[a]	11.3 ± 4.0	4.9-21.2
Fat mass (%)[a]	17.7 ± 5.8	8.1-32.3
Hormone contraceptive use (n - %)	10	66.7
RE experience (y)	7 ± 5	2-16
RE frequency (sessions·wk^{-1})	4 ± 1	2-5
Squat 1RM (kg)[b]	97 ± 13	75-115
Squat 1RM (kg·bw^{-1})	1.5 ± 0.2	1.2-1.8
Bench press 1RM (kg)[b]	66 ± 10	50-82
Bench press 1RM (kg·bw^{-1})	1.0 ± 0.2	0.8-1.3
Energy (kcal)[c]	2208 ± 509	1473-3497
Protein (g·d^{-1})[c]	143 ± 37	67-210
Carbohydrate (g·d^{-1})[c]	209 ± 54	130-301
Fat (g·d^{-1})[c]	84 ± 39	40-182
Caffeine (mg·d^{-1})[d]	341 ± 184	54-692

Note: Range: min-max.

Abbreviations: 1RM, one-repetition maximum; RE, resistance exercise.

[a]Measured with InBody720.

[b]Based on the maximal 1RM across the two familiarizations.

[c]Mean habitual intakes from a 24-h food diary prior to each test day.

[d]Habitual caffeine intake questionnaire.

impedance analysis (InBody 720, InBody Co., Ltd) following (a) 24 hours without vigorous exercise, (b) minimum 2 hours fasting, and (c) emptying the bladder.

Both trials were performed at the same time of day, approximately 1 week after familiarization. The participants performed the caffeine and placebo trials at individually standardized test times, which was self-selected to correspond with the participant's habitual training schedule. The trials were interspersed by 72 hours to ensure treatment washout, allow for recovery and for both trials to be completed within the early follicular phase of the menstruation cycle as previously used by Chen et al[15] This is when the concentration and variation in estrogen and progesterone are lowest as compared to other the phases of the menstrual cycle.[18] Participants using hormone contraceptives were included, as these show very similar levels of estrogen and progesterone to the levels during the early follicular phase.[19] Confirmation of a new menstruation cycle was obtained from each participant prior to confirming trial day 1.

2.3 | Experimental protocol

All participants performed the test battery in the same order each day within the set amount of time of 210 minutes, including rest intervals and breaks, estimated from pilot testing (Figure 1). Upon arrival, participants provided a urine sample for visual assessment of hydration status (The Urine Colour Chart®, Human Hydration, LLC). If the urine color chart indicated a score of 5 or below, the participants were provided 250-500 mL of water to improve hydration levels prior to continuing the protocol. In addition, 4 mL blood was collected from the cubital fossa veins (Vacuette® Multiple use drawing needle; Vacuette® tube, 4 mL K2EDTA, Greiner Bio-One GmbH). Blood was further collected at 60 and 270 minutes following treatment ingestion. Subsequently, height and body mass were measured (SECA stadiometer, Model 213; SECA weight scale 876, respectively). All participants received a standardized meal 45 minutes prior to testing, consisting of 0.4 g·kg^{-1} whey protein powder (0.36 g·kg^{-1} protein) and 1.5 g·kg^{-1} banana (0.35 g·kg^{-1} carbohydrate). All participants performed a standardized warm-up for 10 minutes by cycling on a stationary bicycle at ~100 W at 80-90 RPM (Monark, Ergomedic 828E), followed by a standardized 5 minutes rest, and were equally verbally encouraged to perform to the best of their abilities during all tests. The participants completed questionnaires about their preparation adherence, withdrawal symptoms, and the Brunel Mood score (BRUMS) 24-item questionnaire[31] prior to the protocol and an end-of-trial questionnaire about adverse effects and blinding, where the participants were asked to state if they believed they had received caffeine, placebo or were unsure, after completion of the test battery and final blood sampling.

2.4 | Supplementation

Treatment was given 60 minutes prior to testing, allowing peak plasma levels of caffeine to coincide with testing.[1] The treatments were administered as 150 mL non-caloric Fun Light© cordial concentrate from an opaque bottle. To prepare the caffeine treatment, 4 mg·kg^{-1} anhydrous caffeine (Caffeine, ReagentPlus®, Sigma-Aldrich) was dissolved in the cordial concentrate with heat to ensure complete dissolution of the caffeine. Both treatments were equal in color, taste, and volume due to not diluting the cordial. The drink was rapidly ingested immediately followed by another 150-mL cordial from a separate cup to conceal any potential bitter taste and rinse the mouth of caffeine residues. An independent researcher randomized treatment order, mixed, and administered the treatments and held the key to the randomization until the end of the study.

2.5 | Measurements

2.5.1 | Countermovement jump

Participants performed the countermovement jump (CMJ) to assess jump height (cm), maximal power (W) and

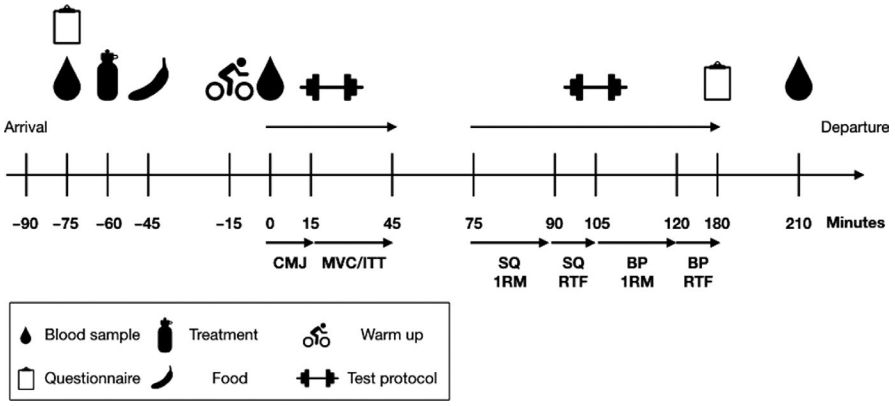

FIGURE 1 Experimental protocol timeline. Overview of the experimental protocol. In addition, urine was observed at arrival for visual hydration status estimation with the urine color chart. 1RM, one-repetition maximum; BP, bench press; CMJ, countermovement jump; ITT, interpolated twitch technique; MVC, maximal voluntary isometric contraction; SQ, squat; RTF, repetitions to failure

maximal force (N). Participants were instructed to stand on a force plate (FP4, HUR Labs OY, Hur AB) with hands kept on their hips with legs shoulder width apart while executing a maximal vertical jump, from an upright position to a self-selected depth immediately prior to jumping. To warm-up, three submaximal CMJ trials with approximately 50%, 75%, and 90% intensity were performed with 1-minute breaks. After another 2 minutes rest, maximal effort CMJ trials with 2 minutes rest between each trial were performed for at least 3 sets. If the third set resulted in an improved jump height compared to the second, the participants were allowed to continue until a set resulted in a decline in performance. Jump height was determined as the center of mass displacement, calculated from take-off force development and force plate-measured body mass with the provided software (Force Platform Software Suite, Version 2.6.51). The single best result was noted and used for statistical analysis. Test-retest measurements revealed a CV of 9.7%, 5%, and 6% for jump height, maximal power, and maximal force, respectively.

2.5.2 | Maximal isometric strength, muscular activation level, and RFD

Peak torque, muscular activation level, and RFD were measured by maximal voluntary isometric contractions (MVC) of the right knee extensor muscles, while seated in a knee extension machine (Knee extension, Gym2000; Software: Acq Knowledge 4.4, Biopac systems Inc) instrumented with a load cell (U2A, Hottinger Baldwin Messtechnik GmbH). The seat was adjusted to 100- and 90- degrees hip and knee flection, respectively, the moment arm pad proximal to the ankle and the knee axis of rotation coincided with that of the apparatus. The participants were strapped across the hip, chest, and ankle of the right leg to minimize any joint movement. Adjustments were recorded to ensure consistent positioning between trials. All participants were instructed to contract as hard and as rapidly as possible. After three submaximal warm-up contractions (~50%, 75%, and 90%), five MVCs were performed with 60 seconds rest intervals. Peak torque, defined as the maximum voluntarily achieved value across the five MVCs, was used in the data analyses. RFD_{max}, defined as the maximum positive change of force over 10 ms intervals from initiation of contraction, as well as torque at 100 ms (from initiation of contraction) was extracted from the software. The recordings had a sampling frequency of 1000 Hz and were smoothed with a moving average of 10 samplings before analyses.

Of the five MVCs, three were un-evoked and two were evoked utilizing the interpolated twitch technique (ITT).[32] The MVCs were performed in an alternating fashion, beginning and ending with an un-evoked contraction. The maximal voluntary activation level across the two attempts is presented. The peak torque of un-evoked MVCs controlled whether the evoked were in fact maximal contractions and contractions with torque prior to stimulus below 80% of peak torque were defined as submaximal and excluded from further calculation of activation level (n = 6). Two self-adhesive surface electrodes (Veinoplus, 8 × 13 cm, Oval shape, Ad Rem Technology) were positioned over the quadriceps of the right leg, one proximally and one distally, in a medial-lateral position to target as many muscle bellies as possible. An intensity test was performed in rested state after the warm-up, to determine the stimulus output level for the ITT. The stimuli were given as 200 μs, 400 V single-imposed signals from a digitimer (Digitimer DS7AH HV Constant current stimulator, Digitimer Ltd.), with successive increments until the evoked force amplitude was no larger than the previous. To ensure maximal evoked force, a 10% increase was added to the stimulus output, equating totally to 660-990 mA. Four "singlet" stimulations about 5 seconds apart and one double-imposed stimulus at this output were given as familiarization with the stimuli. The "doublet" was given as a 10 ms, 100 Hz-stimulus (Digitimer DG2A Train/Delay Generator, Digitimer Ltd.) and was used during the evoked MVC.

The MVC was evoked at the peak of contraction, about 0.5 seconds after initiation, and again as the quadriceps had relaxed and the force curve had returned to baseline. The percentage muscle activation level was determined with the following equation[32]:

$$\text{Muscle activation \%} = 100 - \left(\frac{D \times \left(\frac{\text{Mean force}_{\text{MVC pre - stimulus}}}{\text{Peak force}_{\text{MVC}}} \right)}{\text{Peak force}_{\text{Evoked at rest}}} \right) \times 100 \quad (1)$$

where D is the difference between the voluntary and evoked force:

$$D = \text{Peak force}_{\text{Evoked MVC}} - \text{Mean force}_{\text{MVC pre - stimulus}}$$

If submaximal voluntary force was achieved during the evoked contractions, the calculated muscle activation % was corrected by replacing $\text{Peak force}_{\text{MVC}}$ in Equation (1) with the peak force across the un-evoked contractions. Test-retest measurements revealed a CV of 9.7%, 7.1%, and 18.3% for peak torque, muscle activation level, and RFD_{max}, respectively.

2.5.3 | 1-repetition maximum

The participants completed 1-repetition maximum (1RM) in the squat followed by bench press (T-100G, Eleiko Sport). A standardized warm-up was performed consisting of three

sets with gradually increasing load (50-75-90% of maximal familiarization 1RM) and declining number of repetitions (8-4-1). After 2 minutes rest, the first attempt was performed at 95% of maximal familiarization 1RM. After each successful attempt and 3-minute rest periods, the load was increased by 0.5%-5% (smallest increment 0.5 kg) until the participant reached voluntary failure. If the lift was unsuccessful, the load was decreased (0.5%-5%) for another attempt until 1RM was determined. The bench press 1RM test was performed in the same manner with a preceding 5-minute rest following the squat RTF test (Figure 1). A Smith rack was used to prevent substantial change in the technique during the squats. Intra-individual control of equipment utilized (limited to weight lifting shoes, belt, wrist support, and knee sleeves), squat stance and bar position, bench press set up, and grip distance that the participants were accustomed to were noted and reproduced in the second trial. The CV for this test was 2.3% for squats and 2.4% for bench press, and number of attempts were 4-6 and 3-5, respectively.

2.5.4 | Muscular endurance and perceived exertion and pain

Repetitions to failure (RTF) were performed with 60% of maximal familiarization 1RM to ensure equal absolute load. The repetitions were counted out loud and a smart phone metronome application (Tap Metronome v1.2.1, Daniel Soper) was set to 15 BPM/4-seconds intervals to standardize the repetitions. The technical requirements were (a) depth equating to hips below parallel and maintaining an upright torso position, and (b) a controlled change of direction and fully extended arms in the top position, for squats and bench press, respectively. If unable to complete a repetition within the two metronome signals, the following repetition had to be completed in time, otherwise the previous repetition was counted as the last. Failure was otherwise defined as failure to complete the repetition at all. The CV for this test was 2.0% for squats and 2.4% for bench press.

From pilot testing and previous studies at 60% of 1RM,[25,27] it was expected that the participants would complete over 20 repetitions in both the squat and bench press RTF test. Following repetition 10, the participants gave ratings of perceived exertion from the 11-point Borg RPE C-10 scale (0 [rest] to 10 [maximal exertion]). Perceived pain was rated from the 11-point NRS perceived pain scale (0 [no pain] to 10 [worst imaginable pain]) immediately after the RTF tests.

2.6 | Plasma analysis

All samples were centrifuged for 10 minutes at 3000 rpm, 1700 *g*, and 4°C (Heraeus Megafuge 16R, ThermoFisher Scientific, Thermo Electron LED GmbH) before transferring plasma to two 1.5 mL micro tubes (MCT-150-C, Axygen, Inc for storage at −80°C until further preparation and analyses.

Samples were analyzed in duplicate with reverse phase LC-MS (Dionex Ultimate HPLC 3000 system; Agilent TOF 6230, positive electrospray ionization [ESI]), based on the method used by Chen et al.[33] We were not able to separate paraxanthine and theophylline; hence, all paraxanthine analyses included small contributions (~4% of total caffeine metabolites concentration) from theophylline.[34] Individually prepared quality control samples at three concentration levels and a blank sample were included in each run of the plasma analyses. Limit of detection (LOD) and limit of quantification (LOQ) were determined based on signal-noise ratio to be <0.008 $\mu g \cdot mL^{-1}$ and <0.05 $\mu g \cdot mL^{-1}$, respectively. In all samples where the analytes were non-detected or estimated <LOQ, values were substituted with worst case scenarios equal to LOD and LOQ, respectively, that is biased high, to enable statistical analyses comparing baseline to 60- and 270 minutes.

2.7 | Statistical analyses

The sample size was calculated using a priori t tests for paired samples to ensure sufficient statistical power in the main analyses (G*Power version 3.1, Heinrich-Heine University).[35] With α-level set at 0.05 for the main outcomes and a 1-β error probability of 0.8, we used the mean and SD from Goldstein et al[27] to calculate the sample size. Ten participants were needed to detect a true mean difference in 1RM strength of 0.8 kg (1.54% difference). Due to an expected drop out of 25%, we aimed to recruit a minimum of 15 subjects for the present study.

All variables' distributions were tested with the Shapiro-Wilks normality test and assessing skewness, kurtosis, and histograms. Paired sample t tests and Wilcoxon signed-rank tests were performed on paired differences with Gaussian and non-Gaussian distribution, respectively, and $P < .05$ was considered statistically significant. Values are given as mean ± SD and median (confidence interval) for parametric and non-parametric tests, respectively. To assess "practical" significance, Hedge's g values were calculated with weighted and pooled SD's and adjustment for samples $n < 50$. Effect size cutoffs were defined as <0.25, 0.25-0.5, 0.5-1.0, and >1.0 for trivial, small, moderate, and large effect sizes, respectively.[36] Values are given as mean ± SD and as median (confidence interval) for parametric and non-parametric tests, respectively. The ergogenic effects of caffeine dependent of order of trials and caffeine identification were assessed with unpaired t tests. Pearson r correlation was assessed between habitual caffeine intakes and delta caffeine effects. CV for the main outcomes was calculated from the two familiarizations

and the last familiarization and the placebo trial, and the largest was consistently chosen throughout. Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, Inc).

3 | RESULTS

There were no significant differences in the macronutrient intake prior to each of the trials (carbohydrate [$P = .39$], fat [$P = .62$], protein [$P = .59$]) and overall energy intake ($P = .77$), or in withdrawal symptoms (all $P > .16$) or BRUMS mood score on commencement of either trials (all $P > .42$). On the post-trial question about which treatment the participants thought they received, seven participants (44%) correctly guessed the treatment order (ie, correctly guessed both conditions), stating restlessness, heart palpitations, and or increased energy and motivation as reasons for guessing caffeine. However, 10 participants (66%) total correctly identified caffeine independent of identifying placebo. No differences were observed in the effects of caffeine between the identifiers and non-identifiers of the caffeine condition (all $P > .20$, see Appendix Table A1) or by the order of trials (all $P > .13$, see Appendix Table A2). All performance and plasma caffeine concentration data are shown in Tables 2 and 3, respectively.

3.1 | Countermovement jump

The mean CMJ jump height, maximal power, and maximal force across the two trials were 33 ± 2 cm, 2893 ± 74 W, and 1570 ± 26 N, respectively. Jump height and peak power increased by 2.3 ± 1.1 cm (7.6 ± 4.0%) and 105 ± 63 W (3.8 ± 2.2%), respectively (Table 2; Figure 3). No difference was observed in peak force.

3.2 | Maximal isometric strength, rate of force development, and muscle activation level

The mean peak torque, RFD_{max}, and activation level across the two trials were 177 ± 6 Nm, 19 ± 1 Nm·10 ms^{-1}, and 86 ± 1% muscle activation, respectively. Caffeine significantly increased peak torque of the knee extensors by 11 Nm (CI: 2-18 Nm), corresponding to 4.6 ± 7.3%, compared to placebo (Figure 2). No difference was observed with caffeine on muscle activation level (−2 ± 4%, n = 9, Figure 2), RFDmax (1.1 ± 4.9 Nm·10 ms^{-1} [9.2 ± 26.5%], Figure 3), or torque at 100 ms (−2.9 ± 26.2 Nm, Table 2). Six participants, in one or both of the trials, had a substantially lower force output during the evoked MVC than the unevoked MVC. The force output during the evoked MVC was 26%-78% of the peak torque contraction in these six participants, whom were excluded from the statistical analyses.

TABLE 2 The effect of caffeine on performance outcomes

Performance outcomes	Placebo	Caffeine	Mean of Δ ± SD	95% CI	*P*-value	Effect size- Magnitude
CMJ jump height (cm)	32.0 ± 4.7	34.3 ± 4.5	2.3 ± 1.1	1.7, 2.9	<.001	0.44 - Small
CMJ peak power (W)	2840 ± 430	2946 ± 430	105 ± 63	71, 140	<.0001	0.21 - Trivial
CMJ peak force (N)	1550 ± 247	1588 ± 247	37 ± 96	−16, 91	.16	0.13 - Trivial
MVC peak torque (Nm)	173 ± 29	181 ± 31	11[a]	2, 18	.02	0.23 - Trivial
MVC activation level (%) [n = 9]	87 ± 5	85 ± 5	−2 ± 4	−5, 1	.16	−0.35 - Small
MVC RFD_{max} (Nm·10 ms^{-1})	15 ± 5	17 ± 6	2 ± 5	−0.5, 4.5	.10	0.34 - Small
MVC $Torque_{100ms}$ (Nm)	75 ± 24	72 ± 29	−3 ± 26	−17, 12	.67	−0.09 - Trivial
1RM Squat (kg)	96 ± 14	100 ± 13	4 ± 1	3, 5	<.001	0.27 - Small
RTF Squat (repetitions)	39 ± 17	45 ± 17	5.8 ± 6.2	2, 9	.003	0.27 - Small
RPE Squat rep 10	6 ± 1	6 ± 1	−1[a]	−1, 1	.67	0.05 - Trivial
PP Post-squat	8 ± 1	9 ± 2	0[a]	−1, 0	.60	0.07 - Trivial
1RM Bench press (kg)	66 ± 10	68 ± 11	2 ± 1	2, 3	<.001	0.18 - Trivial
RTF Bench press (repetitions)	21 ± 6	23 ± 6	2 ± 3	0, 3	.01	0.27 - Small
RPE Bench press rep 10	7 ± 1	7 ± 1	0[a]	−1, 1	>.99	0.09 - Trivial
PP Post-bench press	8 ± 2	7 ± 1	0[a]	−1, 0	.14	0.27 - Small

Note: Values are presented as mean ± SD or median[a] and 95% confidence intervals.

Abbreviations: 1RM, one repletion maximum; CI, 95% confidence interval; CMJ, countermovement jump; Δ, difference between trials; MVC, maximal voluntary contractions; PP, perceived pain; RPE, rating of perceived exertion; RTF, repetitions to failure.

[a]Non-Gaussian distributed paired differences tested with Wilcoxon paired rank test.

TABLE 3 The effect of caffeine on plasma concentrations

	Caffeine			Placebo		
Analyte	**Baseline**	**60 min**	**270 min**	**Baseline**	**60 min**	**270 min**
Caffeine ($\mu g \cdot mL^{-1}$)	0.0 ± 0.1	3.6 ± 0.8[a,c]	3.1 ± 0.9[a,b,c]	0.1 ± 0.2	0.1 ± 0.1	0.0 ± 0.0
Paraxanthine ($\mu g \cdot mL^{-1}$)	0.1 ± 0.1	0.8 ± 0.4[a,c]	1.7 ± 0.8[a,b,c]	0.1 ± 0.2	0.1 ± 0.1	0.2 ± 0.2
Theobromine ($\mu g \cdot mL^{-1}$)	0.0 ± 0.0	0.5 ± 0.1[a]	0.7 ± 0.1[a,b]	0.2 ± 0.3[c]	0.6 ± 0.4[a]	0.8 ± 0.4[a]
TC ($\mu g \cdot mL^{-1}$)	0.2 ± 0.3	4.9 ± 0.9[a,c]	5.6 ± 1.0[a,b,c]	0.4 ± 0.6[c]	0.8 ± 0.6	1.0 ± 0.6[a]

Note: All baseline and placebo mean values are based on several substituted values for non-detected and non-quantifiable measurements equal to limit of detection and limit of quantification, respectively, and thus, should be interpreted with caution. Paraxanthine concentrations include a small contribution of the metabolite theophylline.

Values are presented as mean ± SD.

Abbreviation: TC, total concentration of metabolites.

[a]Different from within condition baseline ($P < .05$).

[b]Different from within condition 60 min ($P < .05$).

[c]Different from between condition corresponding time-point ($P < .05$).

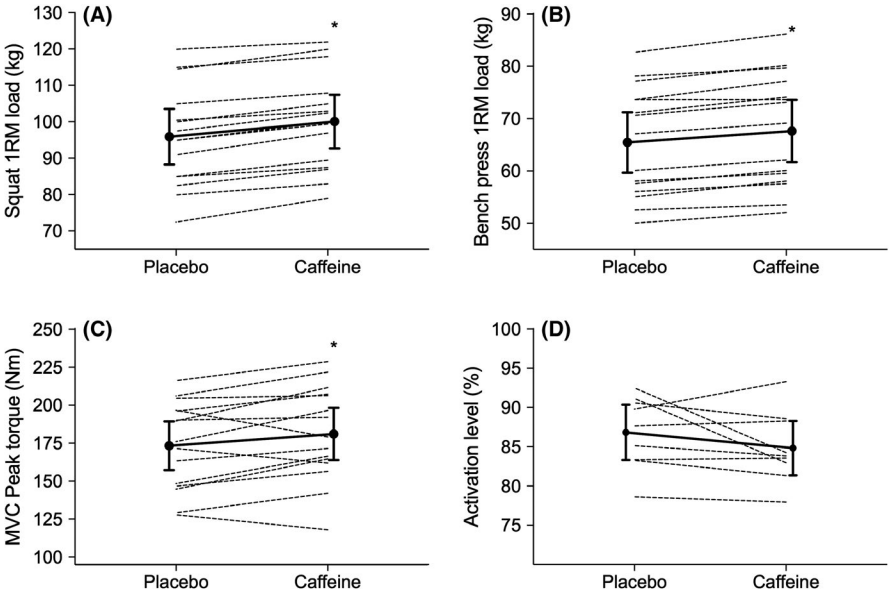

FIGURE 2 Effect of caffeine on maximal strength and activation level. Individual results (dotted lines) and mean ± CI (solid lines) are presented for (A) squats; and (B) bench press 1RM; (C) MVC peak torque and (D) MVC activation level of the knee extensors (n = 9). *Significantly different from placebo ($P < .05$). CI, 95% confidence interval; MVC, maximal isometric voluntary contraction

3.3 | 1-repetition maximum

The mean absolute weight lifted across the two trials was 98.4 ± 2.4 kg and 66.6 ± 1.5 kg for squat and bench press, respectively. Compared to placebo, caffeine ingestion increased 1RM in the squat and in the bench press by 4.1 ± 1.4 kg (4.5 ± 1.9%) and by 2.2 ± 1.0 kg (3.3 ± 1.4%) (see Table 2 and Figure 2).

3.4 | Muscular endurance and perceived effort and pain

The mean absolute weight lifted during the RTF test (60% of familiarization 1RM) was 58 ± 8 kg and 39 ± 6 kg in squats and bench press, respectively. Caffeine significantly increased squat RTF by 5.8 ± 6.2 repetitions (15.9 ± 17.9%) and bench press RTF by 1.8 ± 2.5 repetitions (9.8 ± 13.6%), compared to placebo (Table 2; Figure 4). No differences between trials were found in intra-set RPE at repetition 10 or in at-failure perceived pain (Table 2).

3.5 | Plasma caffeine concentration

Upon arrival on both trial days, plasma caffeine concentrations were negligible, that is, not detected or <LOQ in all participants except two in the placebo trial and one in the caffeine trial (all 0.4 $\mu g \cdot mL^{-1}$). At baseline, theobromine was significantly higher in the placebo compared to the caffeine trial ($P = .03$); however, 8 and 9 of the individual values, respectively, were below LOQ. Due to the chocolate protein powder administered all participants, theobromine was significantly increased from baseline to 60 and

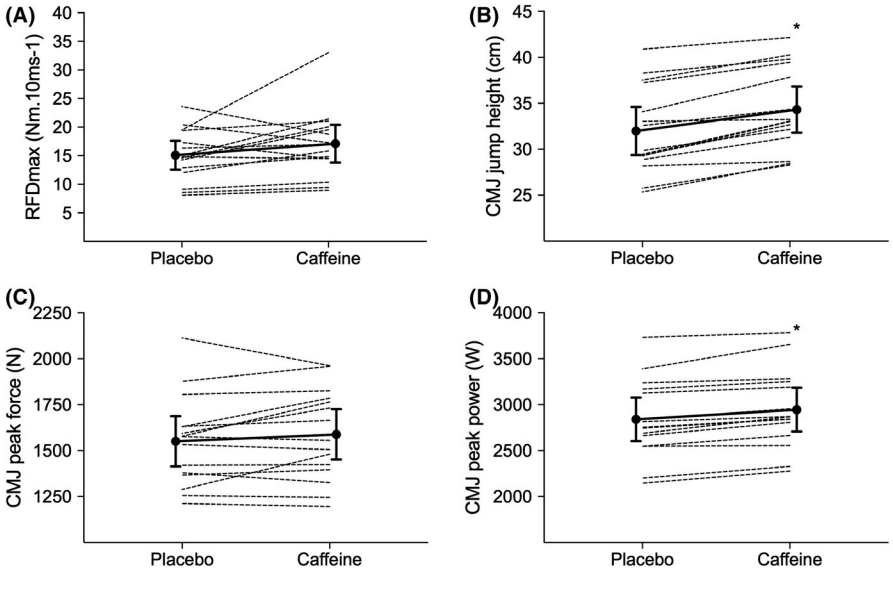

FIGURE 3 Effect of caffeine on rate of force development and countermovement jumps. Individual results (dotted lines) and mean ± CI (solid lines) are presented for (A) RFD max during MVC of the knee extensors; (B) CMJ jump height; (C) CMJ Peak force; and (D) CMJ Peak power. *Significantly different from placebo ($P < .05$). CI, 95% confidence interval; CMJ, countermovement jump; MVC, maximal isometric voluntary contraction; RFD, rate of force development

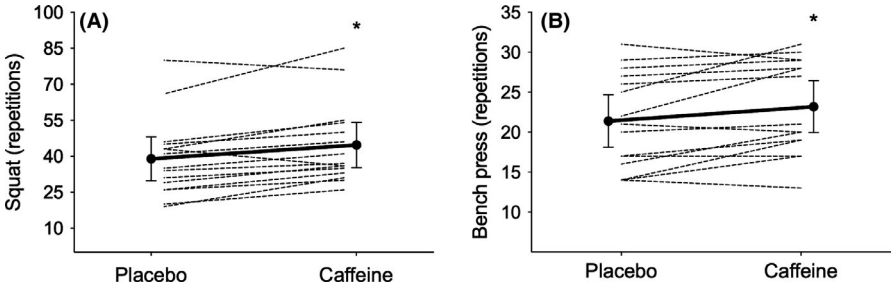

FIGURE 4 Effect of caffeine on muscular endurance. Individual results (dotted lines) and mean ± CI (solid lines) are presented for (A) squats and (B) bench press repetitions to failure at 60% of familiarization-1RM. *Significantly different from placebo ($P < .05$). CI, 95% confidence interval

270 minutes during both trials (all $P < .01$) with no differences between trials ($P > .05$). No other analyte increased from baseline during the placebo trial (all $P > .05$). In the caffeine trial, plasma caffeine concentration increased to 3.6 ± 0.8 ($P < .001$) and 3.1 ± 0.9 $\mu g \cdot mL^{-1}$ ($P < .001$) 60 and 270 minutes following ingestion, respectively, confirming intention to treat (Table 3). Paraxanthine and total metabolite concentration significantly increased from baseline to 60 minutes and 270 minutes following caffeine ingestion (all $P > .001$, Table 3).

3.6 | Habituation

The habitual caffeine intake was 341 ± 184 $mg \cdot d^{-1}$, corresponding to 5.4 ± 2.9 $mg \cdot kg^{-1}$, while the administered dose of 4 $mg \cdot kg^{-1}$ equated to 254 ± 20 mg. The participants were moderate to high caffeine consumers (n categorized as low, moderate, high: 2, 5, 8, respectively). Only the effect of caffeine on muscular endurance was significantly correlated with the habitual intakes (Pearson $r = .52$, $P = .045$ and $r = .58$, $P = .024$ for squat and bench press RTF, respectively).

4 | DISCUSSION

This study investigated the acute effects of 4 $mg \cdot kg^{-1}$ caffeine ingestion on maximal isometric and dynamic muscle strength, power, activation level, RFD, and muscular endurance in resistance-trained females during the early follicular phase. There were several notable findings in the present study. Caffeine ingestion increased dynamic strength measured as 1RM in squat and bench press and isometric knee extension torque, leg muscle power and jump height in CMJ, and improved both squat and bench press muscular endurance measured as repetitions performed until failure at 60% of 1RM. However, no effect of caffeine was observed on RFD, muscle activation, or affect perceived exertion and pain.

In this study, caffeine increased maximal upper body strength, which is in agreement with Grgic et al's recent meta-analysis,[4] as well as the study by Goldstein et al[27] who found increased bench press 1RM performance (1.5%) in 15 resistance-trained females. It is suggested that smaller upper body muscles are less affected by caffeine than larger lower body muscles,[6] which has been implied by studies on for example elbow flexors, not showing effects on maximal strength with

caffeine.[23] Moreover, the positive associations seen between strength and muscle activation with caffeine suggests that muscles with high baseline activation level, such as upper body muscles like the elbow flexors, would likely be less affected by caffeine, that is,there is less room to improve.[6] However, in studies examining multi-joint upper body exercises, there seems to be an overall trend that caffeine has positive effects on strength.[4] This discrepancy might be explained by more muscle mass being recruited as compared to single joint arm exercises, including several muscles with varying activation levels, which might potentiate the effect of caffeine. The present results support that multi-joint upper body strength is indeed affected by caffeine, although possibly still less than lower body strength (3.3% [ES:0.20] vs 4.5% [ES: 0.25] increase in bench press and squat 1RM, respectively).

A novel finding of this study was that a dose of only 4 $mg \cdot kg^{-1}$ caffeine induced a similar or even greater effect on bench press 1RM than a dose of 6 $mg \cdot kg^{-1}$ in the study by Goldstein et al[27] (+3.3% vs +1.5%, respectively). The slight difference in performance between our study and Goldstein et al[27] may partly be explained by severity of adverse events occurring during the caffeine trial. Three participants felt "shaky" and the remaining participants reported no adverse events in the present study, as opposed to three participants "exhibiting intense emotional responses" in the study by Goldstein et al,[27] who reported habitual caffeine intakes of only 0-41 $mg \cdot d^{-1}$. The difference in side effects may be explained by the lower acute dose of caffeine (4 vs 6 $mg \cdot kg^{-1}$) and possibly due to higher habitual caffeine intakes in the present trial (341 $\pm$ 184 $mg \cdot d^{-1}$).

Even though habitual caffeine intake may influence the prevalence of adverse events, it might not affect exercise performance. A study,[37] although on endurance performance, found that acutely ingesting 6 $mg \cdot kg^{-1}$ caffeine increased performance irrespective of whether the daily habitual intake was low (0.8 $mg \cdot kg^{-1}$), moderate (1.9 $mg \cdot kg^{-1}$), or high (4.6 $mg \cdot kg^{-1}$) and that habituation was not correlated with performance. This is in line with the results of the present study, and in addition, and contrary to the above study, we report the same for participants habitually consuming more than the acute dose administered (4 $mg \cdot kg^{-1}$ vs 5.4 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively). Importantly, habitual caffeine may be consumed in small doses over the day, so an acute dose of 4 $mg \cdot kg^{-1}$ may induce higher peak plasma concentration levels than many habitual consumers will experience by administering 5.4 $mg \cdot kg^{-1} \cdot d^{-1}$ daily. This raises the question if the use of high doses is necessary to achieve an equally or potentially better ergogenic effect as seen in the example with Goldstein et al's study.[27] Thus, future research should explore optimal caffeine dosage in relation to habituation.

Squat 1RM increased (+4.5%) significantly in this study, as opposed to Grgic et al's meta-analysis,[4] who observed no overall effect on lower body maximal strength. However, very few studies have been conducted examining dynamic, multi-joint maximal strength in females, indicated by only three included in the above meta-analysis from 2018.[25,27,38] Two of the three studies investigated lower body maximal strength, in which one observed an effect of caffeine and the other a trend of increased performance.[25,38] Thus, one could speculate whether females could have a greater effect of caffeine on lower body dynamic strength compared to males. Furthermore, Grgic et al[4] discuss that the included studies did not report the reliability of their strength tests. In the present study, we report a low CV (2.3%) for the squat 1RM, which could partly explain why we were able to detect an effect of caffeine.

Although no sex differences have been reported on the ergogenic effects of caffeine on exercise performance,[2] only two studies,[15,16] to our knowledge, have investigated caffeine's effects on sex differences with strength-power modalities, showing similar effects (or lack of effects) of caffeine in both males and females.[15,16] As previously mentioned, fluctuating hormone levels with the phases of the menstrual cycles can alter caffeine metabolization speeds,[12] as well as neuromuscular function,[20] and ultimately the ergogenic effects of caffeine. As an example, a recently published study showed that half squat velocity was increased by 1.4%, 5%, and 5.3% in the early follicular, late follicular, and mid-luteal phase, respectively.[17] Thus, ensuring caffeine research in females is conducted during the same menstrual cycle phase is important and furthermore, which phase could potentially affect the effect size. Moreover, only one[15] of the two studies comparing effects of caffeine on strength performance between the sexes controlled for menstruation cycle phase. Therefore, further research is still warranted to establish whether sex differences in ergogenic effect of caffeine on maximal strength occur.

The effect of caffeine on maximal isometric strength observed in this study is in agreement with Warren et al's meta-analysis findings,[6] who found caffeine to have a moderate effect on isometric knee extensor strength. On the other hand, Ali et al[14] found no effects of 6 $mg \cdot kg^{-1}$ caffeine on knee extensor isometric strength in women. However, their protocol measured maximal muscle strength between fatiguing blocks of sprints and consequently, might have masked a caffeine-induced effect on maximal strength.

No effect of caffeine on voluntary muscle activation of the knee extensors was observed in the present study. Previous studies such as Behrens et al[22] demonstrated that strength enhancements by caffeine are associated with increased voluntary activation, and the meta-analysis by Warren et al[6] showed that caffeine has an moderate effect on voluntary muscle activation. On the other hand, Meyers & Cafarelli[39] found no effect of caffeine on muscle activation level after ingesting 6 $mg \cdot kg^{-1}$ of caffeine. The initial

activation level in Meyers & Cafarelli's[39] study was ~94% compared to 70%-80% in the study by Behrens et al,[22] which may suggest that baseline muscle activation level may affect the results, that is, the higher baseline level the less room to improve. In the present study, the participants had a muscle activation level of 85%-87%, which could partly explain why we did not detect any effects of caffeine. Six participants (excluded from analysis of activation level) found it especially difficult to maximally contract during the ITT compared to the un-evoked contractions, independent of treatment. These participants' maximal force output when knowing they would be stimulated was ~25%-75% lower than the intra-trial peak force, although they reported that they felt they were contracting as forcefully as possible. Thus, there may be a psychological factor (ie, being afraid of the electrical stimuli) inhibiting the voluntary contraction when knowing electrical stimuli would be given. Potentially, this might be overcome with further familiarizations to increase the reliability of the test, that is, more than two as in the present study. However, this is a well-known negative effect of stimulus anticipation in the ITT method.[40]

The main mechanism by which caffeine induces ergogenic effects on muscular strength and power is thought to involve supra-spinally-driven increases in muscle activation.[14] Surprisingly, we did not observe any difference between conditions in muscle activation level or RFD_{max}, despite demonstrating effects in 1RM strength, isometric strength, and power. However, the high CV revealed especially for RFD_{max} (18.3%) in the present study increases the risk of a type II error as the statistical power might have been too low to detect a possible effect. Nevertheless, this is a common challenge and even higher CVs than demonstrated in this study are typically reported for RFD in the literature.[41] Furthermore, RFD is closer associated to the rate of muscle activation (RMA) rather than just muscle activation per se, as demonstrated by a recent study showing that the preceding effective motor neuron drive to the muscle influences changes in RFD.[42] Unfortunately, we did not measure RMA in the present study. It could be speculated that the influence of caffeine on changes in RMA is not as profound as with other strength-power measures.

In parallel to the observed effect on muscle strength but in contrast to the lacking effect on RFD, caffeine ingestion improved performance and power measures in the CMJ; the participants jumped 2.3 cm higher with caffeine than in the placebo trial. In line with previous divergent results of caffeine effects on maximal strength, the acute effects of caffeine ingestion on strength-power performance and RFD are inconsistent, but most studies show significant increased lower body power during countermovement jumps.[4] In a subgroup meta-analysis,[4] training status indicated a significant effect for athletes, but not for non-athletes. Although our participants were not athletes, one could speculate that the training status of our participants might have contributed to the positive effect of caffeine. Altogether, the evidence suggests that caffeine acutely improves power, which is in line with our results.

Finally, 4 $mg \cdot kg^{-1}$ caffeine ingestion also significantly increased muscular endurance in both lower (~16%) and upper body (~10%,) exercises in this study. These results are in agreement with Duncan et al,[43] who found 5 $mg \cdot kg^{-1}$ caffeine to increase the number of bench press repetitions to failure (60% of 1RM) in men. On the other hand, these results are in disagreement with other studies in females who did not find any effects on muscular endurance.[16,25,27] However, hormone concentration and hormone contraceptive use were not controlled for[16,25,27] and one did not report familiarization,[16] while the other two only performed one familiarization session.[25,27] In the present study, the participants who performed three familiarization sessions were mainly participants with $CV > 9\%$ in the muscular endurance tests. Hence, there could have been a masking of the caffeine effect in the studies with only one or no familiarization, due to continued learning effect in both trials.

Caffeine reducing pain perception and RPE is a possible mechanism for increased performance,[21] and, as mentioned in the introduction, in a 2005 meta-analysis, Doherty et al,[24] observed that a ~5% reduction in RPE during, as opposed to at-failure, explained about a third of the variance in exhaustive work performance between caffeine and placebo. However, and albeit the analgesic effects might be easier to observe when assessed intra-set compared to at-failure (due to an assumed greater relative difference in motor output between trials when caffeine increases number of repetitions performed), no difference in intra-set RPE was observed between the caffeine and placebo trials in the present study. The fact that RPE was assessed only one time during the set and that a lower dose was used than most of the included studies in the meta-analysis (4 vs 6 $mg \cdot kg^{-1}$) could explain why no difference in intra-set exertion was observed.

Total caffeine concentration and the individual metabolites were significantly higher at 270 minutes as compared to 60 minutes after ingestion, whereas caffeine tended to be lower. Theophylline and paraxanthine can contribute to the pharmacological effect on the central nervous system as these also inhibit the adenosine A_1 and A_2 receptors.[44] Theophylline is considered to be three to five times more potent than caffeine, and paraxanthine may be as potent as caffeine.[44] Thus, we can expect that the participants in the present study had similar effects of caffeine throughout the test protocol (60-270 minutes following ingestion), and we did indeed observe significant effects both on the first (CMJ), as well as the last (bench press RTF) test of the protocol.

Controlling for hormone concentrations in the way which was used in the present study is cost- and time-efficient,

when assuming the participants are having no health issues that would affect their hormones around the menstrual cycle. To our knowledge, this is only the second study on the effects of caffeine on strength performance to control for oscillations in reproductive hormones in this way.[15] However, we did not confirm the hormone concentrations in blood samples, which would be a strength of future studies. Recently, as mentioned, the first study on the effect of caffeine on half squat velocity during three phases of menstrual cycle was published.[17] Nevertheless, we recommend that further studies compare the effects of caffeine on female strength and power performance between the menstrual cycle phases to establish the interaction of female reproductive hormones on the ergogenity of caffeine. This is warranted to further optimize personalized recommendations for caffeine use in female athletes and will inform future research on caffeine in females. Another strength of this study is the blinding efficacy check, a potential bias in the caffeine literature, as recently discussed by Painelli et al[45] and Pickering and Grgic.[46] Although 66% participants correctly guessed when they ingested caffeine, no difference in performance was observed between these and those that guessed incorrectly in the present study. Thus, the performance increments observed in the caffeine trial do not seem to be due to the placebo effect.

A limitation of this study is a skewed counterbalance of treatment order arising due to dropout after randomization. Consequently, ten participants received placebo and five participants received caffeine in the first trial. However, we could not detect an effect of treatment order. All participants had an effect of caffeine irrespective of order of trial on CMJ jump height and power and on maximal strength, and furthermore, 12 of the 15 participants performed better with caffeine in the muscular endurance and isometric strength tests. Nevertheless, the low statistical power in the analyses of treatment order in the latter outcomes increases the risk of type II error.

In conclusion, ingestion of 4 mg·kg^{-1} caffeine 60 minutes prior to tests improved maximal strength and power in highly resistance-trained females during the early follicular phase of menstruation. The caffeine supplementation also increased muscular endurance in both upper and lower body exercises without differences in perceived exertion or pain. Furthermore, very few adverse events were reported, and caffeine-induced ergogenic effects were observed although the participants habitually were consuming in excess of the acute dose.

4.1 | Perspectives

These findings of 3%-5% improvement on maximal strength and power could potentially be relevant to female strength and power athletes, where the margins between top placements in competition can be small. However, within-individual differences in performance need to be taken into account and the acute effects of caffeine may be smaller in a competitive context due to increased arousal. Performance effects of caffeine during the different menstrual cycle phases should be investigated further. Establishing whether menstrual cycle phase affects the ergogenity of caffeine allows optimization of personalized recommendations and will inform future caffeine research. Furthermore, further examination of the potential sex differences in the ergogenic effect of caffeine on strength and power is warranted. At the time being, such research should take into account the effects of menstrual cycle phase. Lastly, the long-term effects of chronic caffeine supplementation on resistance exercise adaptations have not been investigated and are thus warranted.

ACKNOWLEDGEMENT

All authors declare no conflict of interest. No funding was received for this study. The authors would like to thank Dr Hans Kristian Stadheim for the preparation, blinding and administering of the treatments, and to all the research participants for partaking in this study.

AUTHOR CONTRIBUTIONS

MN, LCR, TB, LD, and TR involved in conception and design. MN, LCR, TB, POR, MB, and TR involved in acquisition of data, and/or analysis and interpretation of data. MN and LCR drafted the manuscript. MN, LCR, TB, LD, POR, MB, and TR revised the manuscript.

ORCID

Linn Christin Risvang https://orcid.org/0000-0003-4532-2129
Thomas Bjørnsen https://orcid.org/0000-0002-4010-8038
Lygeri Dimitriou https://orcid.org/0000-0002-5093-558X
Per Ola Rønning https://orcid.org/0000-0003-1341-3229
Truls Raastad https://orcid.org/0000-0002-2567-3004

REFERENCES

1. Del Coso J, Muñoz G, Muñoz-Guerra J. Prevalence of caffeine use in elite athletes following its removal from the World Anti-Doping Agency list of banned substances. *Appl Physiol Nutr Metab*. 2011;36(4):555-561. https://doi.org/10.1139/h11-052
2. Pickering C, Grgic J. Caffeine and exercise: what next? *Sports Med Auckl NZ*. 2019;49(7):1007-1030. https://doi.org/10.1007/s40279-019-01101-0
3. Grgic J. Caffeine ingestion enhances Wingate performance: a meta-analysis. *Eur J Sport Sci*. 2018;18(2):219-225. https://doi.org/10.1080/17461391.2017.1394371
4. Grgic J, Trexler ET, Lazinica B, Pedisic Z. Effects of caffeine intake on muscle strength and power: a systematic review and

meta-analysis. *J Int Soc Sports Nutr*. 2018;15:11. https://doi.org/10.1186/s12970-018-0216-0

5. Grgic J, Grgic I, Pickering C, Schoenfeld BJ, Bishop DJ, Pedisic Z. Wake up and smell the coffee: caffeine supplementation and exercise performance – an umbrella review of 21 published meta-analyses. *Br J Sports Med*. 2020;54(11):681-688. https://doi.org/10.1136/bjsports-2018-100278
6. Warren GL, Park ND, Maresca RD, McKibans KI, Millard-Stafford ML. Effect of caffeine ingestion on muscular strength and endurance: a meta-analysis. *Med Sci Sports Exerc*. 2010;42(7):1375-1387. https://doi.org/10.1249/MSS.0b013e3181cabbd8
7. Polito MD, Souza DB, Casonatto J, Farinatti P. Acute effect of caffeine consumption on isotonic muscular strength and endurance: A systematic review and meta-analysis. *Sci Sports*. 2016;31(3):119-128. https://doi.org/10.1016/j.scispo.2016.01.006
8. Duchateau J, Baudry S. Insights into the neural control of eccentric contractions. *J Appl Physiol*. 2014;116(11):1418-1425. https://doi.org/10.1152/japplphysiol.00002.2013
9. Salinero JJ, Lara B, Jiménez-Ormeño E, et al. More research is necessary to establish the ergogenic effect of caffeine in female athletes. *Nutrients*. 2019;11(7): https://doi.org/10.3390/nu11071600
10. Rietveld EC, Broekman MMM, Houben JJG, Eskes TKAB, van Rossum JM. Rapid onset of an increase in caffeine residence time in young women due to oral contraceptive steroids. *Eur J Clin Pharmacol*. 1984;26(3):371-373. https://doi.org/10.1007/BF00548769
11. de Jonge XAKJ, Boot CRL, Thom JM, Ruell PA, Thompson MW. The influence of menstrual cycle phase on skeletal muscle contractile characteristics in humans. *J Physiol*. 2001;530(Pt 1):161-166. https://doi.org/10.1111/j.1469-7793.2001.0161m.x
12. Lane JD, Steege JF, Rupp SL, Kuhn CM. Menstrual cycle effects on caffeine elimination in the human female. *Eur J Clin Pharmacol*. 1992;43.
13. Pallavi L, D Souza UJ, Shivaprakash G. Assessment of musculoskeletal strength and levels of fatigue during different phases of menstrual cycle in young adults. *J Clin Diagn Res JCDR*. 2017;11(2):CC11-CC13. https://doi.org/10.7860/JCDR/2017/24316.9408
14. Ali A, O'Donnell J, Foskett A, Rutherfurd-Markwick K. The influence of caffeine ingestion on strength and power performance in female team-sport players. *J Int Soc Sports Nutr*. 2016;13(1):46. https://doi.org/10.1186/s12970-016-0157-4
15. Chen H-Y, Wang H-S, Tung K, Chao H-H. Effects of gender difference and caffeine supplementation on anaerobic muscle performance. *Int J Sports Med*. 2015;36(12):974-978. https://doi.org/10.1055/s-0035-1550048
16. Sabblah S, Dixon D, Bottoms L. Sex differences on the acute effects of caffeine on maximal strength and muscular endurance. *Comp Exerc Physiol*. 2015;11(2):89-94. https://doi.org/10.3920/CEP150010
17. Romero-Moraleda B, Del Coso J, Gutiérrez-Hellín J, Lara B. The effect of caffeine on the velocity of half-squat exercise during the menstrual cycle: a randomized controlled trial. *Nutrients*. 2019;11(11):2662. https://doi.org/10.3390/nu11112662
18. Elliott KJ, Cable NT, Reilly T, Diver MJ. Effect of menstrual cycle phase on the concentration of bioavailable 17-β oestradiol and testosterone and muscle strength. *Clin Sci*. 2003;105(6):663-669. https://doi.org/10.1042/CS20020360
19. Kuhl H, Gahn G, Romberg G, März W, Taubert H-D. A randomized cross-over comparison of two low-dose oral contraceptives upon hormonal and metabolic parameters: I. Effects upon sexual hormone levels . *Contraception*. 1985;31(6):583-593. https://doi.org/10.1016/0010-7824(85)90058-7
20. Ansdell P, Brownstein CG, Škarabot J, et al. Menstrual cycle associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic females . *J Appl Physiol*. 2019;126(6):1701-1712.
21. Tarnopolsky MA. Effect of caffeine on the neuromuscular system – potential as an ergogenic aid. *Appl Physiol Nutr Metab*. 2008;33(6):1284-1289. https://doi.org/10.1139/H08-121
22. Behrens M, Mau-Moeller A, Weippert M, et al. Caffeine-induced increase in voluntary activation and strength of the quadriceps muscle during isometric, concentric and eccentric contractions. *Sci Rep*. 2015;5:10209. https://doi.org/10.1038/srep10209
23. Trevino MA, Coburn JW, Brown LE, Judelson DA, Malek MH. Acute effects of caffeine on strength and muscle activation of the elbow flexors. *J Strength Cond Res*. 2015;29(2):513-520. https://doi.org/10.1519/JSC.0000000000000625
24. Doherty M, Smith PM. Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. *Scand J Med Sci Sports*. 2005;15(2):69-78. https://doi.org/10.1111/j.1600-0838.2005.00445.x
25. Arazi H, Hoseinihaji M, Eghbali E. The effects of different doses of caffeine on performance, rating of perceived exertion and pain perception in teenagers female karate athletes. *Braz J Pharm Sci*. 2016;52:685-692.
26. Black CD, Waddell DE, Gonglach AR. Caffeine's ergogenic effects on cycling: neuromuscular and perceptual factors. *Med Sci Sports Exerc*. 2015;47(6):1145-1158. https://doi.org/10.1249/MSS.0000000000000513
27. Goldstein E, Jacobs PL, Whitehurst M, Penhollow T, Antonio J. Caffeine enhances upper body strength in resistance-trained women. *J Int Soc Sports Nutr*. 2010;7:18. https://doi.org/10.1186/1550-2783-7-18
28. Astorino TA, Rohmann RL, Firth K. Effect of caffeine ingestion on one-repetition maximum muscular strength. *Eur J Appl Physiol*. 2008;102(2):127-132. https://doi.org/10.1007/s00421-007-0557-x
29. Wilk M, Krzysztofik M, Filip A, Zajac A, Del Coso J. The effects of high doses of caffeine on maximal strength and muscular endurance in athletes habituated to caffeine. *Nutrients*. 2019;11(8):1912. https://doi.org/10.3390/nu11081912
30. Addicott MA, Yang LL, Peiffer AM, Laurienti PJ. Methodological considerations for the quantification of self-reported caffeine use. *Psychopharmacology*. 2009;203(3):571-578. https://doi.org/10.1007/s00213-008-1403-5
31. Lan MF, Lane AM, Roy J, Hanin NA. Validity of the Brunel mood scale for use with Malaysian athletes. *J Sports Sci Med*. 2012;11(1):131-135.
32. Strojnik V, Komi PV. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. *J Appl Physiol*. 1998;84(1):344-350. https://doi.org/10.1152/jappl.1998.84.1.344
33. Chen F, Hu Z-Y, Parker RB, Laizure SC. Measurement of caffeine and its three primary metabolites in human plasma by HPLC-ESI-MS/MS and clinical application. *Biomed Chromatogr*. 2017;31(6):e3900. https://doi.org/10.1002/bmc.3900
34. Kalow W, Tang BK. The use of caffeine for enzyme assays: a critical appraisal. *Clin Pharmacol Ther*. 1993;53(5):503-514.
35. Faul F, Erdfelder E, Lang A-G, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and

biomedical sciences. *Behav Res Methods*. 2007;39(2):175-191. https://doi.org/10.3758/BF03193146
36. Rhea MR. Determining the magnitude of treatment effects in strength training research through the use of the effect size. *J Strength Cond Res*. 2004;18(4):918-920. https://doi.org/10.1519/14403.1
37. Gonçalves LDS, Painelli VDS, Yamaguchi G, et al. Dispelling the myth that habitual caffeine consumption influences the performance response to acute caffeine supplementation. *J Appl Physiol*. 2017;123(1):213-220. https://doi.org/10.1152/japplphysiol.00260.2017
38. Fett CA, Magalhães Aquino N, Schantz J, Brandão CF, de Araújo Cavalcanti JD, Rezende Fett WC. Performance of muscle strength and fatigue tolerance in young trained women supplemented with caffeine. *J Sports Med Phys Fitness*. 2018;58(3):249-255.
39. Meyers BM, Cafarelli E. Caffeine increases time to fatigue by maintaining force and not by altering firing rates during submaximal isometric contractions. *J Appl Physiol*. 2005;99(3):1056-1063. https://doi.org/10.1152/japplphysiol.00937.2004
40. Button DC, Behm DG. The effect of stimulus anticipation on the interpolated twitch technique. *J Sports Sci Med*. 2008;7(4):520-524.
41. Milivoj D, Jelena I. Metrological characteristics of different early isometric rate of force development characteristics of leg extensors. *MOJ Sports Med*. 2017;1(3):1. https://doi.org/10.15406/mojsm.2017.1.00013
42. Del Vecchio A, Negro F, Holobar A, et al. You are as fast as your motor neurons: speed of recruitment and maximal discharge of motor neurons determine the maximal rate of force development in humans. *J Physiol*. 2019;597(9):2445-2456.
43. Duncan MJ, Oxford SW. The effect of caffeine ingestion on mood state and bench press performance to failure. *J Strength Cond Res*. 2011;25(1):178-185. https://doi.org/10.1519/JSC.0b013e318201bddb
44. Fredholm BB, Bättig K, Holmén J, Nehlig A, Zvartau EE. Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev*. 1999;51(1):83.
45. De Salles PV, Brietzke C, Franco-Alvarenga PE, Canestri R, Vinícius Í, Pires FO. Comment on: "caffeine and exercise: what next?" *Sports Med*. 2020;50(6):1211-1218.
46. Pickering C, Grgic J. Authors' reply to Painelli et al.: comment on "caffeine and exercise: what next?" *Sports Med*. 2020;50(6):1219-1221. https://doi.org/10.1007/s40279-020-01279-8

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Norum M, Risvang LC, Bjørnsen T, et al. Caffeine increases strength and power performance in resistance-trained females during early follicular phase. *Scand J Med Sci Sports*. 2020;00:1–14. https://doi.org/10.1111/sms.13776